

Wild Rice Study - Hydroponic Tests

Dose response analysis

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Objective

To model the change in wild rice growth to increasing concentrations of total sulfide. To use a dose response model to estimate EC5, EC10, EC20, and EC50 values.

Data

- 3 Hydroponic Tests (Range Finder, Definitive 1, Definitive 2)
- 5 treatments per test (Control, T1, T2, T3, T4)
- 3 replicates per treatment
- Independent variables (measured by the University of MN, Duluth)
 - mean initial total sulfide
 - time weighted arithmetic mean total sulfide
 - time weighted geometric mean total sulfide
 - time weighted mean final total sulfide

-All censored data were substituted with half the detection limit ($22.1/2 = 11.05 \text{ ug/L}$)

-Response variables

- Change in plant mass = final – initial in units of mg
- Relative change in plant mass = $(\text{change in plant mass} - \text{min change in plant mass}) / (\text{max change in plant mass} - \text{min change in plant mass})$. This was calculated separately for each test, but values were pooled in a single analysis across all three tests.

Analysis

I fit dose response curves using a 4-parameter logistic model using R software and the drc package (suggested by reviewer Mark Hanson). These analyses use change in plant mass as the response variable, which provides a better indication of whole-plant growth change (compared to length change) and does not assume exponential growth was occurring (growth rate constant makes this assumption). I ran analyses using the 4 different sulfide measures and 2 different response measures described above (suggested by reviewers). 95% confidence intervals of the model fits are shown in figures below (suggested by reviewer Curt Pollman).

General 4-Parameter Logistic Model

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- B = slope parameter, indicating the speed with which the curve rises between min and max responses.
- C = right-side horizontal asymptote (min response)
- D = left-side horizontal asymptote (max response)
- E = x value at the inflection point of the curve.

EC values

- EC values are calculated for positive values of y. For example, $\text{EC5} = 0.95*D$, $\text{EC20} = 0.8*D$.
- To calculate EC values, the above equation can be rearranged to solve for x = $\exp(\ln(E) + \ln((D-C)/(y-C) - 1)/B)$
- Additional work is required to calculate the uncertainty around these EC estimates. I still need to follow up on Russ Erickson's advice on how to do this. Reviewer Mark Hanson suggested using the drc package in R to do these calculations. Functions in this package allow EC values and their respective confidence intervals to be calculated based on the full range of response values. These are fairly straight forward calculations and I applied this approach to calculate EC values for the analyses using relative weight change. However, for all other data sets we calculate EC values based on positive response values only when the full range includes negative growth. It is less straight forward how to calculate confidence intervals for EC values calculated in this way.

Summary

Results depend on the measure of sulfide used. The largest EC values were estimated using mean initial sulfide, followed by time weighted arithmetic mean sulfide, time weighted geometric mean sulfide, and time weighted mean final sulfide. The 95% confidence intervals and p-values for fitted model parameters show that the models with the greatest uncertainty are those that use data from the Definitive 2 test and models that use mean final sulfide as the independent variable. The model fits using data pooled across all three tests provide the most robust model parameter estimates.

Table of EC values

Test	Sulfide measure	EC5 (ug/L)	EC10 (ug/L)	EC20 (ug/L)	EC50 (ug/L)
Rangefinder	Mean initial sulfide	111	161	240	459
Rangefinder	Time weighted arithmetic mean sulfide	32	56	102	267
Rangefinder	Time weighted geometric mean sulfide	4	10	26	122
Rangefinder	Time weighted mean final sulfide	0	0	0	1
Definitive1	Mean initial sulfide	120	157	210	327
Definitive1	Time weighted arithmetic mean sulfide	31	50	83	178
Definitive1	Time weighted geometric mean sulfide	3	6	17	74
Definitive1	Time weighted mean final sulfide	0	0	0	6
Definitive2	Mean initial sulfide	278	298	322	365
Definitive2	Time weighted arithmetic mean sulfide	142	156	172	202
Definitive2	Time weighted geometric mean sulfide	69	71	73	77
Definitive2	Time weighted mean final sulfide	11	14	19	32
All 3 tests	Mean initial sulfide	269	292	319	375
All 3 tests	Time weighted arithmetic mean sulfide	144	157	173	207
All 3 tests	Time weighted geometric mean sulfide	35	53	82	178
All 3 tests	Time weighted mean final sulfide	1	3	8	50
All 3 tests*	Mean initial sulfide	265 (183-347)	287 (230-344)	313 (282-345)	363 (292-436)
All 3 tests*	Time weighted arithmetic mean sulfide	141 (97-186)	154 (122-187)	170 (150-190)	200 (164-237)
All 3 tests*	Time weighted geometric mean sulfide	34 (3-64)	51 (18-84)	78 (41-115)	165 (62-267)
All 3 tests*	Time weighted mean final sulfide	1 (-6-9)	3 (-13-19)	9 (-26-44)	53 (-52-159)

*EC values (95% C.I.) were calculated using the full range of response variables, instead of based on the maximum asymptote value only (parameter D).

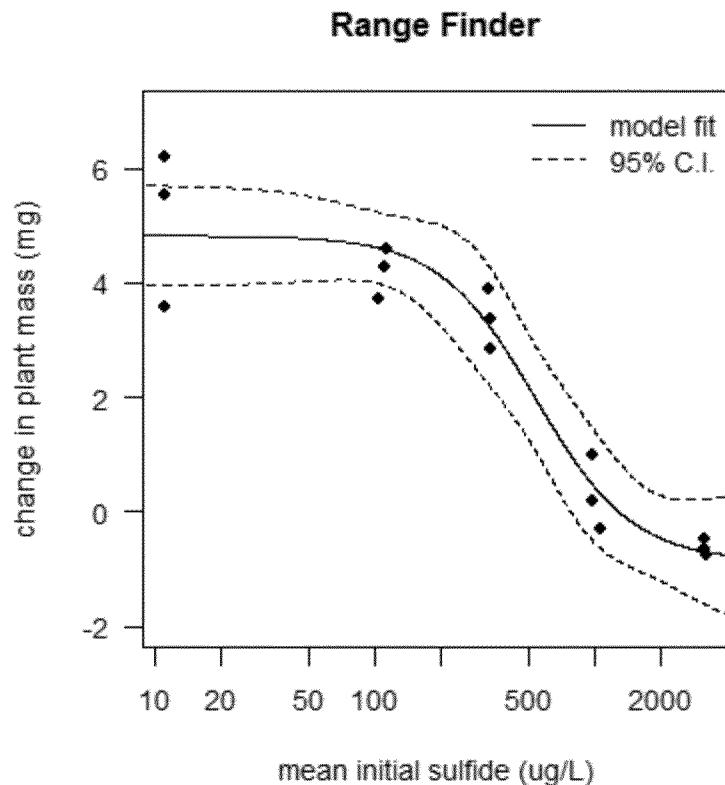
The following pages describe each model used to calculate these EC values in more detail.

Model 1A**Data set = Range Finder****Y = change in plant mass****X = mean initial total sulfide****Censored data = half Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	1.97714	0.84479	2.34038	0.0391
C. Lower Limit:(Intercept)	-0.85580	0.61311	-1.39582	0.1903
D. Upper Limit:(Intercept)	4.82260	0.40117	12.02121	0.0000
E. inflection point:(Intercept)	535.35317	113.11682	4.73275	0.0006

Residual standard error:

0.741181 (11 degrees of freedom)

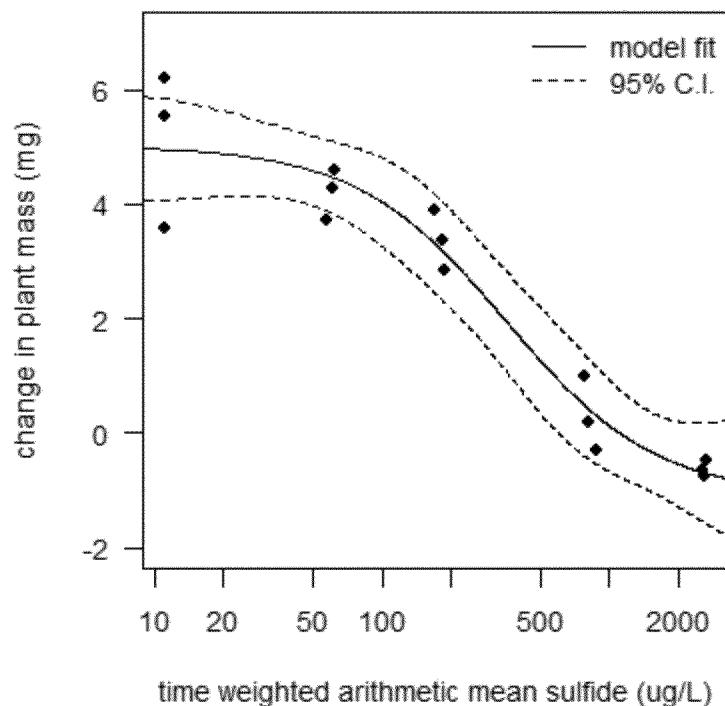


Model 1B**Data set = Range Finder****Y = change in plant mass****X = time weighted arithmetic mean total sulfide****Censored data = half the Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	1.32114	0.54075	2.44316	0.0326
C. Lower Limit:(Intercept)	-1.08623	0.83419	-1.30214	0.2195
D. Upper Limit:(Intercept)	5.01542	0.48804	10.27667	0.0000
E. inflection point:(Intercept)	350.12720	116.18954	3.01341	0.0118

Residual standard error:

0.7121094 (11 degrees of freedom)

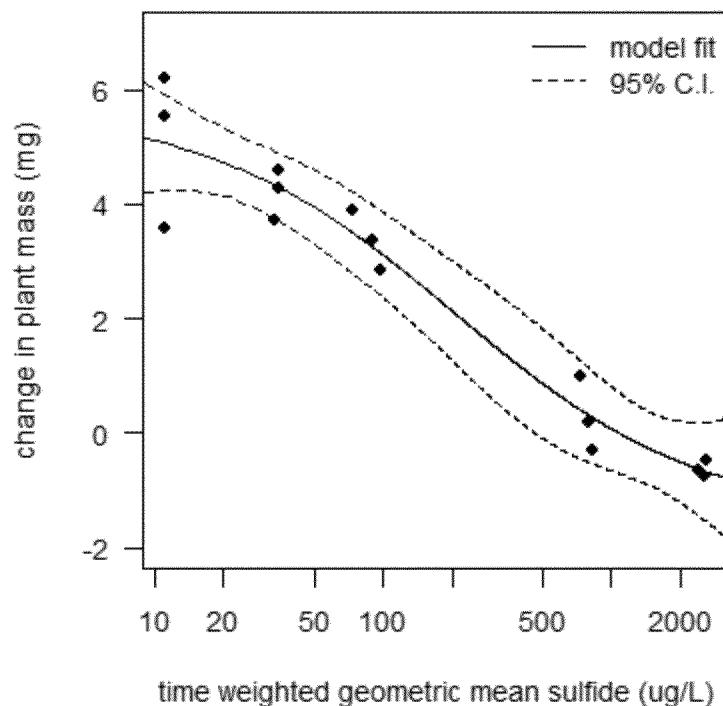
Range Finder

Model 1C**Data set = Range Finder****Y = change in plant mass****X = time weighted geometric mean total sulfide****Censored data = half the Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	0.80519	0.44617	1.80468	0.0985
C. Lower Limit:(Intercept)	-1.49317	1.55485	-0.96033	0.3575
D. Upper Limit:(Intercept)	5.68841	1.15164	4.93941	0.0004
E. inflection point:(Intercept)	206.02195	128.07984	1.60854	0.1360

Residual standard error:

0.6806042 (11 degrees of freedom)

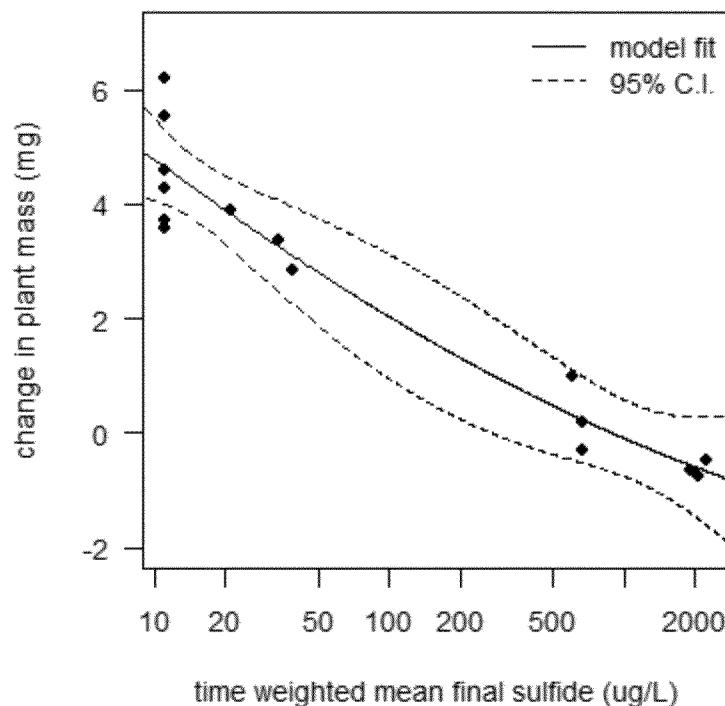
Range Finder

Model 1D**Data set = Range Finder****Y = change in plant mass****X = time weighted mean final total sulfide****Censored data = half the Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	0.27606	0.40851	0.67579	0.5131
C. Lower Limit:(Intercept)	-3.46597	6.53017	-0.53076	0.6061
D. Upper Limit:(Intercept)	15.27539	28.61216	0.53388	0.6040
E. inflection point:(Intercept)	4.15896	37.69093	0.11034	0.9141

Residual standard error:

0.7478122 (11 degrees of freedom)

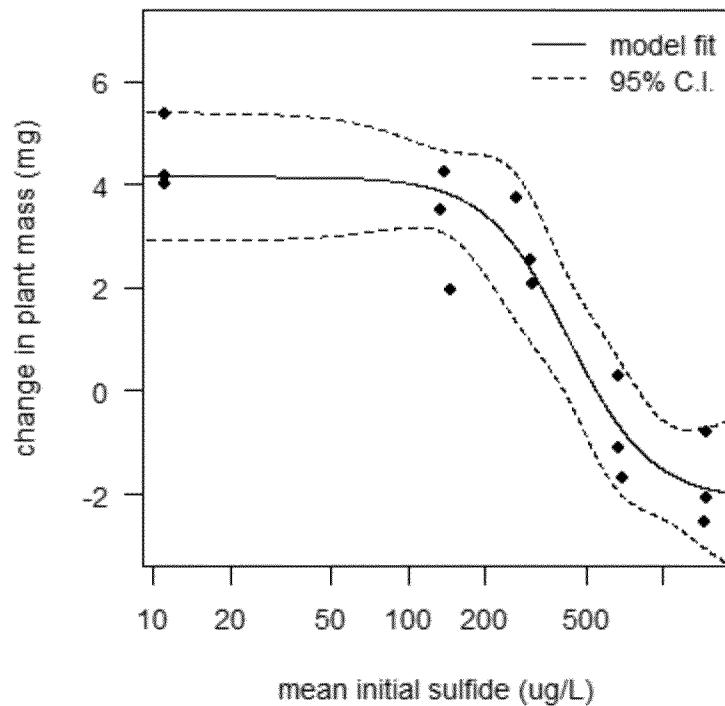
Range Finder

Model 2A**Data set = Definitive 1****Y = change in plant mass****X = mean initial total sulfide****Censored data = half Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	2.66502	1.50373	1.77227	0.1040
C. Lower Limit:(Intercept)	-2.10725	0.87188	-2.41691	0.0342
D. Upper Limit:(Intercept)	4.15279	0.56479	7.35284	0.0000
E. inflection point:(Intercept)	424.78688	79.04416	5.37405	0.0002

Residual standard error:

0.9422625 (11 degrees of freedom)

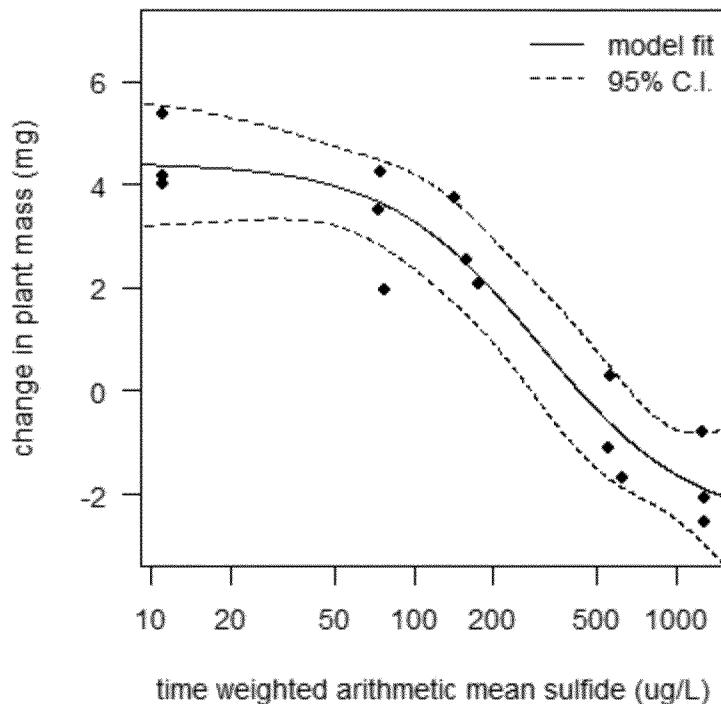
Definitive 1

Model 2B**Data set = Definitive 1****Y = change in plant mass****X = time weighted arithmetic mean total sulfide****Censored data = half Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	1.50205	0.70256	2.13798	0.0558
C. Lower Limit:(Intercept)	-2.61831	1.40407	-1.86480	0.0891
D. Upper Limit:(Intercept)	4.42244	0.60425	7.31885	0.0000
E. inflection point:(Intercept)	300.23481	115.45417	2.60047	0.0247

Residual standard error:

0.8892767 (11 degrees of freedom)

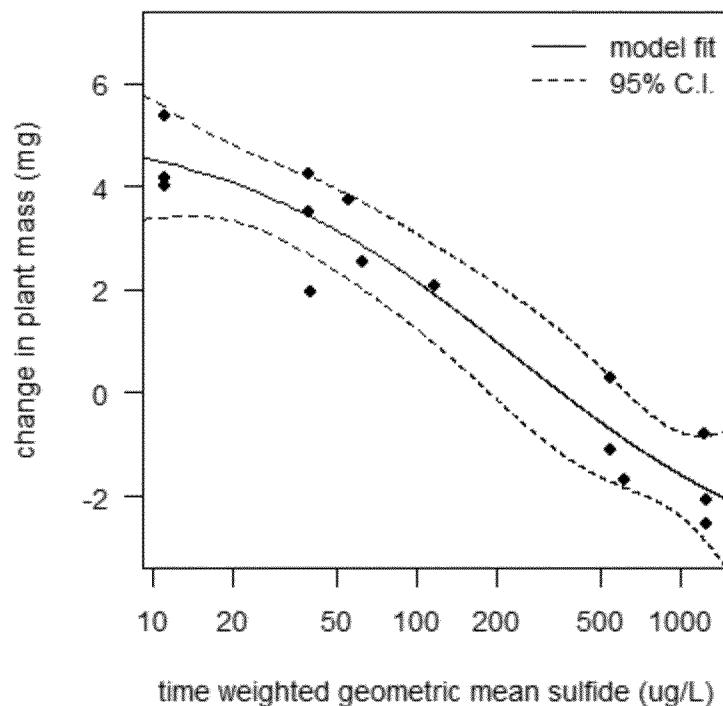
Definitive 1

Model 2C**Data set = Definitive 1****Y = change in plant mass****X = time weighted geometric mean total sulfide****Censored data = half Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	0.77646	0.59748	1.29956	0.2203
C. Lower Limit:(Intercept)	-3.77998	3.98687	-0.94811	0.3634
D. Upper Limit:(Intercept)	5.25131	1.69428	3.09944	0.0101
E. inflection point:(Intercept)	232.13754	254.32758	0.91275	0.3809

Residual standard error:

0.8416099 (11 degrees of freedom)

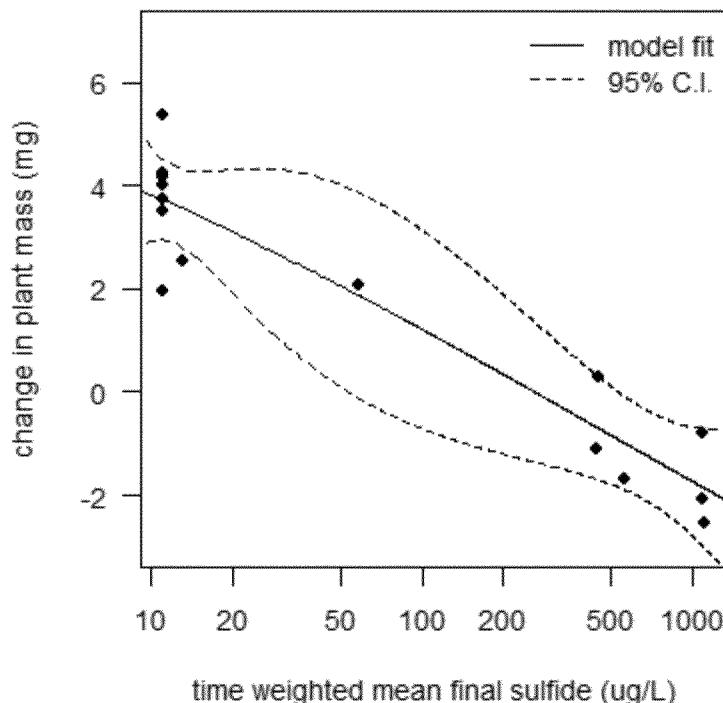
Definitive 1

Model 2D**Data set = Definitive 1****Y = change in plant mass****X = time weighted mean final total sulfide****Censored data = half Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	0.27789	1.22582	0.22670	0.8248
C. Lower Limit:(Intercept)	-9.97863	49.15245	-0.20301	0.8428
D. Upper Limit:(Intercept)	8.70943	31.01740	0.28079	0.7841
E. inflection point:(Intercept)	424.55252	4051.05088	0.10480	0.9184

Residual standard error:

0.9790673 (11 degrees of freedom)

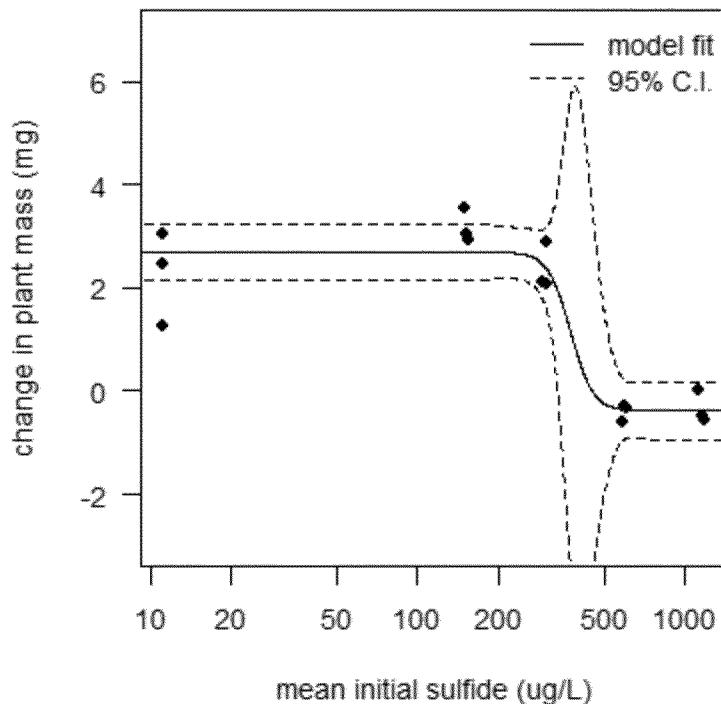
Definitive 1

Model 3A**Data set = Definitive 2****Y = change in plant mass****X = mean initial total sulfide****Censored data = half the Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	10.32719	12.81704	0.80574	0.4375
C. Lower Limit:(Intercept)	-0.38704	0.26113	-1.48215	0.1664
D. Upper Limit:(Intercept)	2.69414	0.24259	11.10596	0.0000
E. inflection point:(Intercept)	374.30075	99.64596	3.75631	0.0032

Residual standard error:

0.5905536 (11 degrees of freedom)

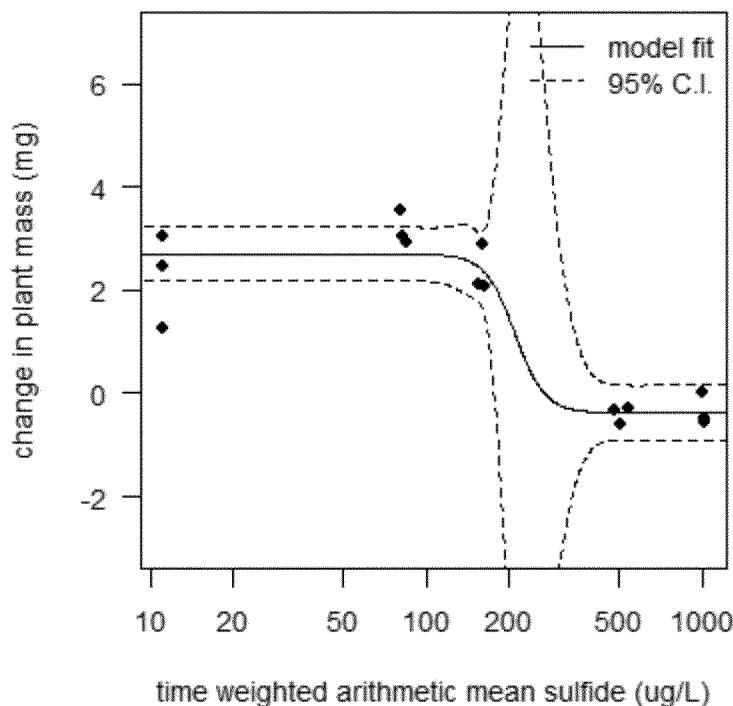
Definitive 2

Model 3B**Data set = Definitive 2****Y = change in plant mass****X = time weighted arithmetic mean total sulfide****Censored data = half the Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	8.05646	15.97367	0.50436	0.6240
C. Lower Limit:(Intercept)	-0.37734	0.24133	-1.56357	0.1462
D. Upper Limit:(Intercept)	2.69914	0.24071	11.21305	0.0000
E. inflection point:(Intercept)	208.66958	114.65200	1.82003	0.0960

Residual standard error:

0.5880628 (11 degrees of freedom)

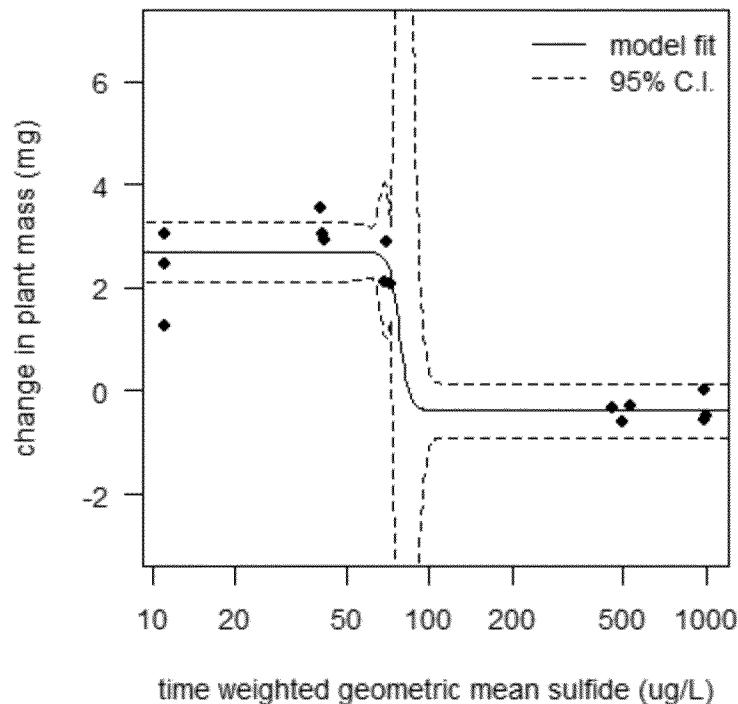
Definitive 2

Model 3C**Data set = Definitive 2****Y = change in plant mass****X = time weighted geometric mean total sulfide****Censored data = half the Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	24.63572	106.74667	0.23079	0.8217
C. Lower Limit:(Intercept)	-0.37636	0.23749	-1.58475	0.1413
D. Upper Limit:(Intercept)	2.69642	0.25898	10.41150	0.0000
E. inflection point:(Intercept)	77.79413	24.09442	3.22872	0.0080

Residual standard error:

0.581754 (11 degrees of freedom)

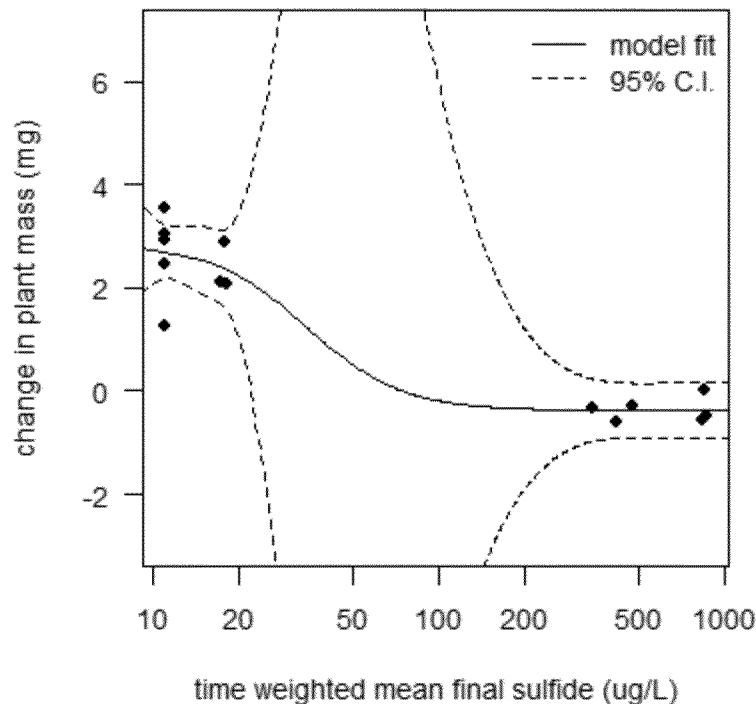
Definitive 2

Model 3D**Data set = Definitive 2****Y = change in plant mass****X = time weighted mean final total sulfide****Censored data = half the Detection Limit value****Parameter estimates:**

	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	2.61678	10.57716	0.24740	0.8092
C. Lower Limit:(Intercept)	-0.37909	0.25757	-1.47179	0.1691
D. Upper Limit:(Intercept)	2.86079	1.26373	2.26376	0.0448
E. inflection point:(Intercept)	34.56099	62.43389	0.55356	0.5910

Residual standard error:

0.5863753 (11 degrees of freedom)

Definitive 2

Model 4A**Data set = All three tests pooled together****Y = change in plant mass****X = mean initial total sulfide****Censored data = half the Detection Limit value****Parameter estimates:**

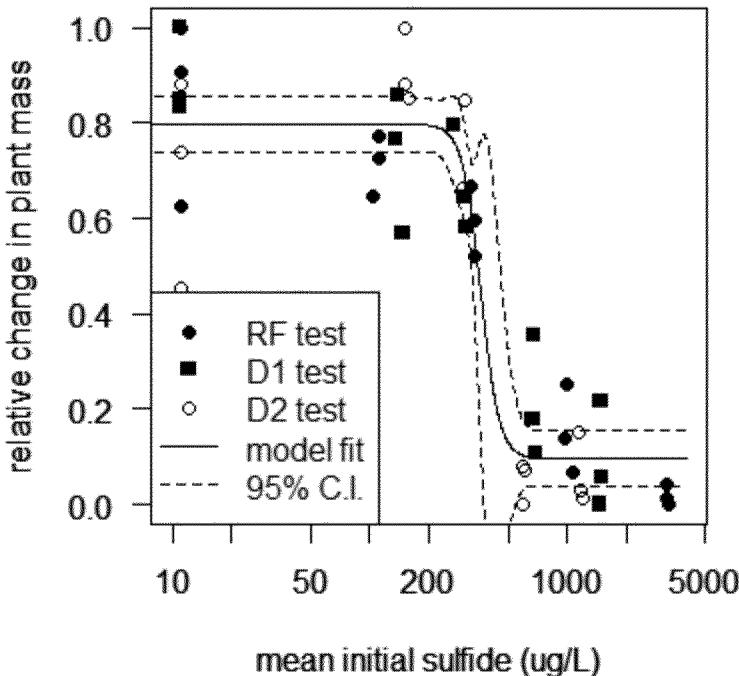
	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	9.319175	6.900684	1.350471	0.1843
C. Lower Limit:(Intercept)	0.096534	0.029732	3.246853	0.0023
D. Upper Limit:(Intercept)	0.797732	0.028770	27.728253	0.0000
E. inflection point:(Intercept)	363.703919	35.607262	10.214319	0.0000

Residual standard error:

0.122186 (41 degrees of freedom)

**Estimated effective doses
(Delta method-based confidence interval(s))**

	Estimate	Std. Error	Lower	Upper
1:5	265.174	40.726	182.926	347.42
1:10	287.311	28.251	230.257	344.36
1:20	313.432	15.796	281.531	345.33
1:50	363.704	35.607	291.794	435.61

All 3 tests

Model 4B**Data set = All three tests pooled together****Y = change in plant mass****X = time weighted arithmetic mean total sulfide****Censored data = half the Detection Limit value****Parameter estimates:**

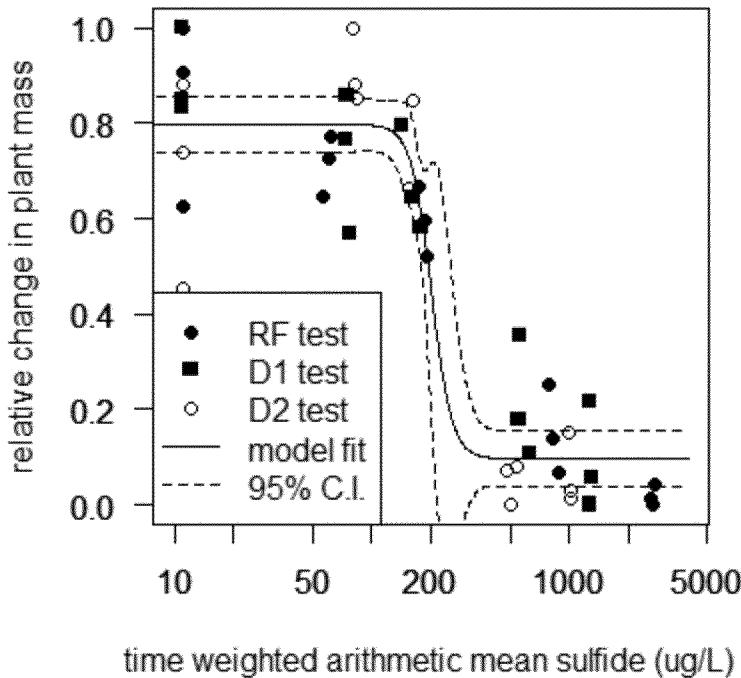
	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	8.436791	5.439690	1.550969	0.1286
C. Lower Limit:(Intercept)	0.096533	0.028491	3.388222	0.0016
D. Upper Limit:(Intercept)	0.797714	0.028431	28.057621	0.0000
E. inflection point:(Intercept)	200.400065	18.152941	11.039537	0.0000

Residual standard error:

0.1208026 (41 degrees of freedom)

**Estimated effective doses
(Delta method-based confidence interval(s))**

	Estimate	Std. Error	Lower	Upper
1:5	141.3608	22.1308	96.6668	186.05
1:10	154.4518	16.0387	122.0609	186.84
1:20	170.0343	9.7177	150.4091	189.66
1:50	200.4001	18.1529	163.7395	237.06

All 3 tests

Model 4C**Data set = All three tests pooled together****Y = change in plant mass****X = time weighted geometric mean total sulfide****Censored data = half the Detection Limit value****Parameter estimates:**

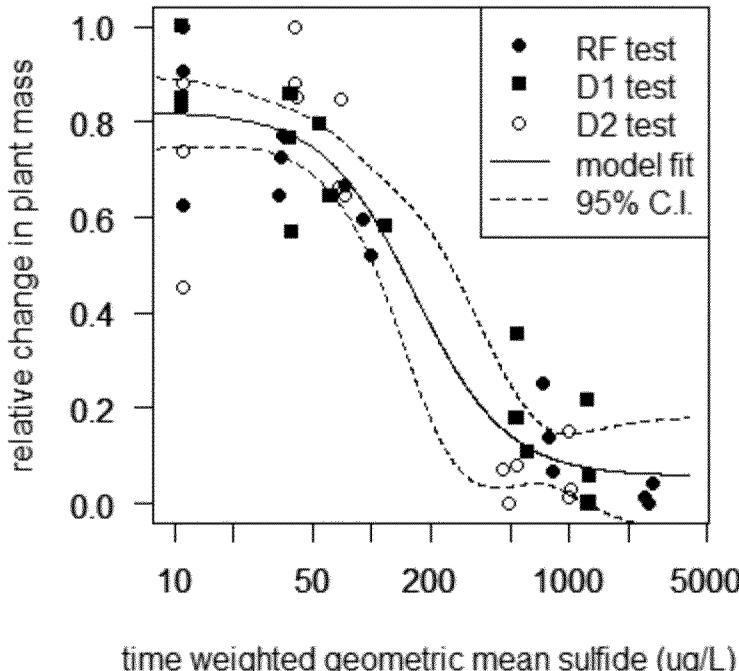
	Estimate	Std. Error	t-value	p-value
B. Slope:(Intercept)	1.862615	0.708387	2.629374	0.0120
C. Lower Limit:(Intercept)	0.057387	0.064542	0.889140	0.3791
D. Upper Limit:(Intercept)	0.822056	0.040017	20.542752	0.0000
E. inflection point:(Intercept)	164.545680	50.906285	3.232325	0.0024

Residual standard error:

0.1208188 (41 degrees of freedom)

Estimated effective doses
(Delta method-based confidence interval(s))

	Estimate	Std. Error	Lower	Upper
1:5	33.8649	15.1085	3.3527	64.377
1:10	50.5793	16.3403	17.5794	83.579
1:20	78.1723	18.2169	41.3825	114.962
1:50	164.5457	50.9063	61.7384	267.353

All 3 tests

Model 4D**Data set = All three tests pooled together****Y = change in plant mass****X = time weighted mean final total sulfide****Censored data = half the Detection Limit value****Parameter estimates:**

		Estimate	Std. Error	t-value	p-value
B.	Slope:(Intercept)	0.765012	0.504005	1.517865	0.1367
C.	Lower Limit:(Intercept)	-0.026247	0.156668	-0.167530	0.8678
D.	Upper Limit:(Intercept)	1.036430	0.392908	2.637844	0.0117
E.	inflection point:(Intercept)	53.478625	52.461303	1.019392	0.3140

Residual standard error:

0.1199688 (41 degrees of freedom)

Estimated effective doses
(Delta method-based confidence interval(s))

	Estimate	Std. Error	Lower	Upper
1:5	1.1393	3.7303	-6.3942	8.6728
1:10	3.0257	8.0204	-13.1718	19.2232
1:20	8.7334	17.3677	-26.3413	43.8082
1:50	53.4786	52.4613	-52.4691	159.4264

All 3 tests